The Effect of Different Heavy Metal Acetate Solutions on the Inhibition of Catalase Enzyme

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The purpose of this experiment was to determine the effect of different aqueous solutions of ionic compounds on the rate of reaction of the enzyme catalase. The hypothesis states that zinc and cadmium acetate solutions will significantly inhibit catalase and its ability to produce oxygen. In the experiment, there were five trials performed with the lead acetate, zinc acetate, acetic acid, cadmium acetate, and distilled water (the last being the control group). In order to calculate the results, the ANOVA single factor test was used, comparing each solution to water. From results of the ANOVA test, it was found that the lead acetate, zinc acetate, and the acetic acid all significantly reduced the rate of reaction of the enzyme catalase, while cadmium acetate did not. This experiment is significant because it has potential applications in finding effective chemical treatments of bacteria that produce catalase.

Introduction

Enzymes are proteins that play an integral role in human body's most basic and complex functions, involved in various catalytic and anabolic reactions. Among the innumerable families of enzymes found in the human body are peroxidases, whose main function is to break down peroxides. Catalase is a peroxidase that catalyzes hydrogen peroxide (H_2O_2) into water and oxygen to rid the body of its toxic effects. The cells of the body create free superoxide oxygen (O_2^-). The oxygen acts as the terminal electron acceptor in the electron transport chain of the aerobic respiration cycle and produces the highly toxic and reactive superoxide with an unpaired electron. The superoxide oxygen is then converted into hydrogen peroxide by superoxide dismutase enzyme.

In commercial and industrial settings, catalase may be introduced to substances that prevent the reaction between the enzyme and its substrate. By studying various aqueous solutions and their effects relating to catalase inhibition, this research may help identify common solutions that would inhibit its function. The independent variable for this study is the different ionic compounds in an aqueous solution. The amount of oxygen produced can be compared to the control group using the compound guaiacol. According to the *AP Biology Investigative Labs*, guaiacol "binds instantly with oxygen to form tetraguaiacol, which is brownish in color. The greater the amount of oxygen produced, the darker brown the solution will become". A colorimeter measured the absorbance at 470 nm wavelength. As the solution darkened with the formation of brown tetraguaiacol, it's optical density increased, implying an increase in enzyme-substrate reaction.

A study by Yang *et al.* studied the effects of cadmium, zinc, and lead on the enzyme activity in soil. The researchers took samples of meadow cinnamon soil, and thoroughly air-dried and sieved the soil of impurities. The researchers then added varying combinations of acetate solutions of the three metals to soil samples. After the solutions were added to the soil, canola seeds were sown, grown, and harvested. The activity of the soil enzymes was measured after the canola plants were harvested. The paper's chemical analysis section states that a potassium permanganate titration method was used to find the concentration of the catalase enzyme, and it appeared to use H_2O_2 as a substrate for the catalase enzyme and an endpoint for the titration. The researchers concluded that of the three metals, cadmium "was the most effective inhibitor" of catalase and the other three enzymes, causing a greater decrease in catalase activity than adding all three metals to the soil (Yang *et al.* 2006).

All aerobic organisms produce catalase enzyme which is controlled by the CAT family of genes. Catalase can be found so easily in organisms because of its inherent defensive properties against superoxides as well as peroxides. There are several known inhibitors of catalase, such as hydrogen cyanide and vitamin C. Sodium arsenate is a known activator, or substance that induces the reaction between substrate and enzyme, for catalase (Kertulis-Tartar *et al.* 2009).

Catalase in the body is used to counteract the harmful effects of oxygen reactivity. When oxygen enters the body, it has a tendency to react with surrounding molecules to form the harmful superoxide and hydrogen peroxide. In the case of hydrogen peroxide, this can decompose into hydroxide ions which, according to the RCSB Protein Data Bank, can mutate DNA. By working with the enzyme superoxide dismutase (or SOD), catalase can help to rid of these toxic chemicals. SOD is known to convert the reactive superoxide radicals into hydrogen peroxide, which are then converted into water and oxygen by catalase. Catalase converts its substrate using two H_2O_2 molecules in two steps. Catalase first removes an oxygen atom from an H_2O_2 molecule, forming water in the process. This same process is done to the second H_2O_2 molecule. The two removed oxygen atoms are bound together through one of the four heme molecules— an iron-centered, ring-shaped molecule— that compose the catalase enzyme, forming O_2 (Goodsell 2004). This process is simply shown in the chemical equation:

$$2H_2O_2 \rightarrow 2H_2O + O_2$$

Alternatively, the hydrogen peroxide is broken down into water by a redox reaction with coenzyme NADH by peroxidase. A study by Schwartz *et al.* studied how catalase and SOD worked in *E. coli* to protect the bacteria from superoxide and hydrogen peroxide. Both of these chemicals are produced by leukocytes in the bacterium's host as a "bactericidal mechanism". The experiment tested how phenazine methosulfate could inhibit or activate catalase activity in *E. coli*. The researchers admit later in the paper, however, that the results regarding catalase are likely inaccurate because the enzyme could not— at the time— be isolated from the bacteria. Another issue was that H_2O_2 and superoxide were likely outside the *E. coli*, while the two enzymes were inside it. Because the harmful substrates were separated from the enzymes by the bacteria's plasma membrane and cell wall, the enzymes could only access the outside "after the organism has already been irreversibly damaged". For this reason, it would be better to use catalase that has already been isolated from the bacteria to avoid the variable of the bacteria's health (Schwartz *et al.* 1982).

The use of spectrophotometry on the catalase-peroxide systems is not a new technique. A 1952 paper by Beers and Sizer first proposed this method of measuring catalase activity. The use of spectrophotometry in this manner was found through the application of the Beer-Lambert Law, which states that hydrogen peroxide's optical density, or the degree to which it slows the refraction of light, is directly related to its concentration. With an understanding of this law, the catalyzing of H_2O_2 by catalase will reduce its concentration, thus reducing the optical density. Using an ultraviolet spectrophotometer, readings of optical density were taken every 10 seconds. The procedure included a constant jet propelled the hydrogen peroxide into the cuvette— the container for the object observed in the spectrophotometer— containing the catalase. After the 1.0 mL of H_2O_2 were expelled, a constant stream of purified air blew over the cuvette to remove any oxygen bubbles that would otherwise interfere with the spectrophotometric readings. The results of this experiment states that the hydrogen peroxide optical density and its concentration are correlated

(Beers and Sizer 1952). While the same spectrophotometer will not be used in this study, a possible link between the concentration of the substrate and the readings from the colorimeter can help in interpreting results.

Weydert and Cullen show various assays of antioxidant enzymes such as SOD and catalase. The assay for catalase was based on that of Beer and Sizer to find the amount of peroxide removed. The authors note the limits of catalase, stating that catalase cannot be easily saturated by H_2O_2 and that it will be inactivated if the concentration of H_2O_2 is greater than 0.1 M. Measurement of catalase activity is done using an activity gel in a glass plate, a combination of chemicals including H_2O_2 that forms a blue color where the catalase and its substrate interact. This colors forms because the removal of the peroxide allows for a precipitate to form from two other compounds, potassium ferrocyanide (which is formed from potassium ferricyanide as a side effect of H_2O_2's removal) and ferric chloride. The resulting image on the plate will be of a single blue band that enlarges with increased catalase activity (Weydert and Cullen 2009). While this assay directly measures and depicts catalase activity, the guaiacol-oxygen reaction will be sufficient for this experiment as a measure of catalase activity.

The purpose of this research is to study the effect of different aqueous solutions of ionic compounds on the oxygen produced by catalase. The hypothesis states that, as Yang *et al.* found that heavy metals in soil such as zinc and cadmium will significantly inhibit catalase and its ability to produce oxygen. The null hypothesis states that none of the aqueous solutions of ionic compounds will inhibit the oxygen production from the breakdown of hydrogen peroxide using catalase.

Methods

The solutions studied were cadmium acetate, zinc acetate, lead acetate, and acetic acid, each with a molarity of 0.1 M. Distilled water was used as a control. In addition to these five solutions, a catalase enzyme solution was prepared by grinding 3 g of turnips in 500 mL of distilled water. A 1% solution of hydrogen peroxide and 1% solution of guaiacol was also prepared in this experiment. The materials gathered include equipment from Vernier, specifically a LabView Colorimeter and a Vernier interface, and test tubes and cuvettes for storing solutions. Various conditions were kept constant throughout the experiment, namely the enzyme and substrate concentrations, as well as the relative volumes of each during the reaction.

This LabView Colorimeter was connected to the Vernier interface, which was then connected to a computer running Logger Pro 3. Three test tubes were then labelled, one "B" for the blank solution, another "E" for the enzyme solution, and the third "S" for the substrate solution. The blank solution was prepared by filling test tube B with 2 mL of the solution in use, 1 mL of 1% hydrogen peroxide, 0.5 mL of 1% guaiacol, and 1 mL of phosphate buffer solution. Before being placed into the colorimeter, the cuvettes were wiped with with a non-abrasive tissue. After cleaning the cuvette, the cuvette was filled with 3 mL blank solution. The cuvette was then placed into the colorimeter, making sure not to contaminate the clear walls of the container. These clear sides of the cuvette faced the beam of the colorimeter. The wavelength of the colorimeter's beam was set to 470 nm (Blue) and calibrated according to this wavelength. After calibrating the colorimeter for a beam with wavelength of 470 nm, data-collection was performed using Logger Pro 3. The sample rate was set to 1 sample/second. After setting up the data-collection parameters on Logger Pro 3, the enzyme solution was prepared by filling the tube labelled E with 2 mL of the solution in use, 1 mL enzyme extract, and 1 mL of phosphate buffer solution. The substrate solution was then prepared by filling the test tube labelled S with 1 mL of the solution in use, 2 mL of 1% hydrogen peroxide, and 1 mL of 1% guaiacol. During this time, the substrate solution was added to the enzyme solution and thoroughly mixed together. The cuvette was then filled with 3 mL of the combined solution. The lid of the colorimeter was quickly shut with the cuvette inside of it and data-collection began. This process was performed quickly to fully capture the change in color due to the formation of tetraguaiacol. After data-collection, the graph recorded on Logger Pro was analyzed using a linear fit. This empirically showed the average rate of reaction of catalase in the presence of the particular solution in absorbance/second (abs/s). The cuvette and test tube contents were then discarded and cleaned for next trial or next solution. This process was repeated ten times for each solution for a total of fifty trials.

Safety and Ethical Guidelines

Both hydrogen peroxide and guaiacol are potentially harmful substances. Guaiacol is known to cause skin irritation at high concentrations. Precautions were taken not to come into direct contact with these chemicals, despite the relatively low concentrations used in the experiment.

Results

As expected, the rate of reaction of catalase was greatest in the control solution of distilled water, with an average rate of reaction of 0.0018304 absorbance per second. Following the control, the highest rate of reaction of catalase was in the presence of cadmium acetate, followed by zinc acetate, acetic acid, and lead acetate. Lead acetate, in fact, had a negative rate of reaction, though this may be the result of the precipitate lead peroxide forming in the cuvette during the reaction. This insoluble product likely prevented the 470 nm light from passing through the colorimeter, greatly reducing the absorbance recorded by the LabView Colorimeter. A comparison of the average rates of reaction in the presence of the various aqueous solutions can be found in Figure 1 in Appendix C.

A single factor ANOVA test was performed on the raw data, comparing the results of the heavy metal acetate solutions and the acetic acid to those of the distilled water. It was found that zinc acetate, lead acetate, and acetic acid had significantly the rate of reaction of catalase. Cadmium acetate, however, did not significantly impact the rate of reaction of catalase. The large F-value for acetic acid may be due to the use of a 1.0 M solution, as opposed to the molarity of 0.1 M used for the other solutions. Hence, the data for acetic acid would be skewed due to the relatively high molarity in comparison to the other solutions.

Conclusions

The purpose of this experiment was to find the effect of different aqueous solutions of ionic compounds on the oxygen produced by catalase. Based on the results, it was found that zinc acetate and acetic acid reduced the rate of absorbance when compared to the control, distilled water. This reduction of enzyme activity is likely due to the dissociated acetate ions interfering with the formation of an effective enzyme-substrate complex between catalase and hydrogen peroxide. The negative rate of absorbance caused by the addition of lead acetate is likely due to an error in experimental design. When the lead acetate-based enzyme and substrate solutions were mixed, the precipitate lead peroxide formed, likely blocking the path of the colorimeter's beam. The colorimeter would then interpret this impediment to the beam of light as a sharp reduction in its absorbance, thus the negative rate of absorbance. The results of the cadmium acetate trials were considered statistically insignificant by the ANOVA test, suggesting that the change in the rate of absorbance was negligible. Like the lead acetate reaction, the addition of cadmium acetate would have likely produced a precipitate that would have impeded the path of light in the colorimeter, in this case insoluble cadmium oxide.

The acetic acid appeared to have the most significant impact on the rate of absorbance, and thus the slowest rate of reaction of the catalase. The severe reduction in the rate of reaction and the high F value in the ANOVA test between acetic acid and the control may be due to the

solution's high molarity of 1.0 M when compared to the other solutions with molarities of 0.1 M. Because this solution was the first to be tested, the researchers had assumed a molarity of 1.0 M would be optimal for all of the solutions tested. The researchers then found that the molarity needed to be reduced to properly test the other, more opaque solutions with the colorimeter. Although they reduced the molarity to 0.1 M in other tests, the researchers did not have enough time to test the acetic acid solution again with the lower molarity.

The hypothesis is partially supported in that zinc acetate significantly reduced the rate of reaction of catalase. Cadmium acetate, however, did not significantly reduce the rate of reaction, according to the ANOVA test when compared to distilled water. No reliable conclusions could be drawn regarding lead acetate's effect on the rate of reaction of catalase, as our method didn't account for the formation of the opaque precipitate during experimentation.

Further research can explore other variables that were kept constant in this experiment, such as testing pH, temperature or concentration of either solution. It should be noted that a possible source of error was in the inherent change in pH due to the dissociation of the ionic compounds in the water (acetic acid, for example, would have a lower pH than the distilled water control). Another source of error could include the variability of readings on the colorimeter, thus ultimately altering the data calculated at the end. Alternatively, one could test more solutions and vary the molarity of the solutions for a broader comparison of the solutions' effects of the rate of reaction of catalase.

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Notes and References

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Supplemental Information—Appendix A: Raw Data

Table One - Acetic Acid

Trial	Absorbance/Sec	Correlation	RMSE	Y-Intercept
1	0.0001791	0.9901	0.001483	0.02768
2	0.0001519	0.9876	0.001406	0.05161
3	0.0002165	0.9929	0.001511	0.07459
4	0.0002847	0.9902	0.002342	0.1234
5	0.0002905	0.9842	0.003403	0.02987

Table Two - Cadmium Acetate

Trial	Absorbance/Sec	Correlation	RMSE	Y-Intercept
1	0.003401	0.9999	0.002237	0.04296
2	0.001438	0.8841	0.04433	0.03454
3	0.001136	0.9463	0.02262	0.4869
4	0.0001573	0.8471	0.005756	0.2215
5	0.0002312	0.827	0.009165	0.02122

Table Three - Lead Acetate

Trial	Absorbance/Sec	Correlation	RMSE	Y-Intercept
1	-0.001055	-0.4687	0.116	0.8991
2	-0.003095	-0.9101	0.08218	0.6837
3	-0.002231	-0.8699	0.07708	0.4511
4	-0.0009644	-0.8211	0.0391	0.3835
5	-0.0006924	-0.8433	0.02574	0.6141

Table Four - Zinc Acetate

Trial	Absorbance/Sec	Correlation	RMSE	Y-Intercept
1	0.0008953	0.9769	0.01142	0.2707
2	0.0006756	0.9854	0.006796	0.3972
3	0.0009119	0.9776	0.01146	0.1358
4	0.0002296	0.7187	0.01295	0.01295
5	0.0003148	0.8386	0.01193	0.3715

Table Five - Distilled Water (Control)

Trial	Absorbance/Sec	Correlation	RMSE	Y-Intercept
1	0.001631	0.975	0.02169	0.2443
2	0.001886	0.981	0.02194	0.2513
3	0.001615	0.9792	0.01949	0.2454
4	0.002116	0.9768	0.02707	0.2416
5	0.001904	0.9854	0.0171	0.2373

Supplemental Information—Appendix B: ANOVA Test, Solutions compared to Distilled Water (Control)

Zinc Acetate

Anova: Single Factor		Zinc Acetate with Water				
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
Column 1	5	0.0030272	0.00060544	0.000000102146573		
Column 2	5	0.009152	0.0018304	0.000000044 0583		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.000003751317504	1	0.000003751317504	51.3159025	0.00009581272316	5.317655063
Within Groups	0.000000584819492	8	0.000000073 1024365			
Total	0.000004336136996	9				

Lead Acetate

Anova: Single Factor		Lead Acetate with Water				
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
Column 1	5	-0.0080378	-0.00160756	0.000001039412588		
Column 2	5	0.009152	0.0018304	0.000000044 0583		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.000029548 9224	1	0.000029548 9224	54.54493098	0.00007726393603	5.317655063
Within Groups	0.000004333883552	8	0.000000541735444			
Total	0.000033882805960	9				

Graphical Analysis and Descriptive Statistics

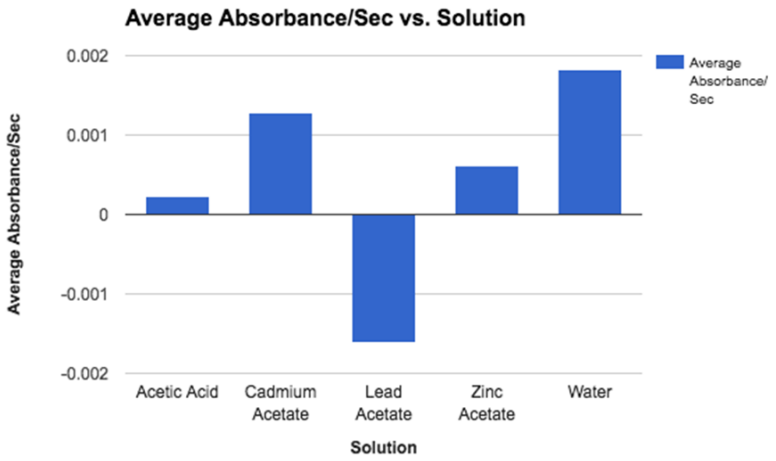

Figure 1: Bar Graph of Average Absorbance/Sec for Each Solution

Descriptive Statistics

	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Mean	Std Dev	Median
Zinc Acetate	8.953E-04	6.756E-04	9.119E-04	2.296E-04	3.148E-04	6.054E-04	3.196E-04	6.756E-04
Lead Acetate	-1.055E-03	-3.095E-03	-2.231E-03	-9.644E-04	-6.924E-04	-1.608E-03	1.020E-03	-1.055E-03
Cadmium Acetate	3.401E-03	1.438E-03	1.136E-03	1.573E-04	2.312E-04	1.273E-03	1.314E-03	1.136E-03
Acetic Acid	1.791E-04	1.519E-04	2.165E-04	2.847E-04	2.905E-04	2.245E-04	6.200E-05	2.165E-04
Distilled Water, Control	1.631E-03	1.886E-03	1.615E-03	2.116E-03	1.904E-03	1.830E-03	2.099E-04	1.886E-03